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Hepatobiliary

Frequent Mutation of Isocitrate Dehydrogenase *(IDH)1* and *IDH2* in Cholangiocarcinoma Identified Through Broad-Based Tumor Genotyping

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Abstract

Cancers of origin in the gallbladder and bile ducts are rarely curable with current modalities of cancer treatment. Our clinical application of broad-based mutational profiling for patients diagnosed with a gastrointestinal malignancy has led to the novel discovery of mutations in the gene encoding isocitrate dehydrogenase 1 (*IDH1*) in tumors from a subset of patients with cholangiocarcinoma. A total of 287 tumors from gastrointestinal cancer patients (biliary tract, colorectal, gastroesophageal, liver, pancreatic, and small intestine carcinoma) were tested during routine clinical evaluation for 130 site-specific mutations within 15 cancer genes. Mutations were identified within a number of genes, including *KRAS* (35%), *TP53* (22%), *PIK3CA* (10%), *BRAF* (7%), *APC* (6%), *NRAS* (3%), *AKT1* (1%), *CTNNB1* (1%), and *PTEN* (1%). Although mutations in the metabolic enzyme IDH1 were rare in the other common gastrointestinal malignancies in this series (2%), they were found in three tumors (25%) of an initial series of 12 biliary tract carcinomas. To better define *IDH1* and *IDH2* mutational status, an additional 75 gallbladder and bile duct cancers were examined. Combining these cohorts of biliary cancers, mutations in *IDH1* and *IDH2* were found only in cholangiocarcinomas of intrahepatic origin (nine of 40, 23%) and in none of the 22 extrahepatic cholangiocarcinomas and none of the 25 gallbladder carcinomas. In an analysis of frozen tissue specimens, *IDH1* mutation was associated with highly elevated tissue levels of the enzymatic product 2-hydroxyglutarate. Thus, *IDH1* mutation is a molecular feature of cholangiocarcinomas of intrahepatic ori-

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gin. These findings define a specific metabolic abnormality in this largely incurable type of gastrointestinal cancer and present a potentially new target for therapy. ***The Oncologist*** 2012;17:72–79

Introduction

High-throughput tumor genotyping is becoming an integral part of the initial clinical management of cancer patients [1, 2]. The ability to direct targeted cancer agents based on tumor-specific molecular abnormalities has had a clear and positive impact on the management of patients with non-small cell lung, breast, colon, skin, and gastrointestinal tumors [3–7]. Evaluating a broad range of key cancer gene mutations across diverse cancers has the potential for identifying new therapeutically relevant mutations. Whereas genetic signatures in colorectal and pancreatic cancers have been well defined [8, 9], this approach can expand our knowledge of less-studied gastrointestinal malignancies such as biliary tract carcinomas.

Biliary tract carcinomas encompass gallbladder carcinoma and bile duct cancer (cholangiocarcinoma). Nearly 12,000 new cases are identified in the U.S. annually and only 10% of patients present with early-stage disease that can be cured through surgical resection [10, 11]. Even with combination cytotoxic chemotherapy, the prognosis for patients remains poor and the median survival duration is ~1 year [12]. New and effective molecularly targeted therapies are urgently needed [13]. Although known mutations in the adenomatous polyposis coli (*APC*), *BRAF*, deleted in pancreatic carcinoma locus 4 (*DPC4*), epidermal growth factor receptor (*EGFR*), Kirstenras (*KRAS*), phosphatidylinositol 3-kinase, catalytic, alpha (*PIK3CA*), and p53 tumor suppressor (*TP53*) genes in cholangiocarcinoma and/or gallbladder carcinoma may help prioritize patient assignment to clinical trials of new drugs [14–19], the full spectrum of relevant mutations across the biliary tract carcinoma subtypes has not been clearly defined.

Somatic mutations in the cytoplasmic and peroxisomal isocitrate dehydrogenase gene *IDH1* and its mitochondrial counterpart *IDH2* have been identified through large genomic screens of human leukemias, glioblastomas, and sarcomas. At the same time, experimental studies have implicated these mutations as driver events in oncogenesis. These mutations cause a single amino acid change at a conserved arginine residue within the isocitrate binding site of *IDH1* (R132) or *IDH2* (R172, R140), resulting in decreased enzymatic activity for oxidative decarboxylation of isocitrate to α-ketoglutarate [20–22]. Although the oncogenic mechanism is not certain, these mutations confer a novel enzymatic function leading to the NADPH-dependent reduction of α-ketoglutarate to the proposed oncometabolite *R*(–)-2-hydroxyglutarate [22–24]. Recurrent *IDH1* and *IDH2* mutations have been identified in a very limited number of tumor types, including ~70% of diffuse or secondary gliomas—including diffuse astrocytomas (grade II), anaplastic astrocytomas (grade III), secondary glioblastomas (grade IV), oligodendrogliomas (grade II), anaplastic oligodendrogliomas (grade III), oligoastrocytomas (grade II), and anaplastic oligoastrocytomas (grade III)—approximately 20% of acute myelogenous leukemia (AML) cases, and, most recently, the majority of central and periosteal cartilaginous tumors [20, 23, 25–36]. Collectively, these findings define a distinct subset of tumor types for which *IDH1* and *IDH2* mutations may provide new insights into pathogenesis and therapeutic targeting.

This study presents the results of mutational profiling of 287 patients across gastrointestinal cancer, identifying for the first time *IDH1* mutations in a significant subset of patients with intrahepatic cholangiocarcinoma. Our findings further highlight the important role of molecular diagnostic testing for discovery of novel mutations of diagnostic and therapeutic relevance.

Materials and Methods

Patients and Samples

Tumors from patients at the Massachusetts General Hospital (MGH) Cancer Center who had been diagnosed with a gastrointestinal malignancy underwent mutational profiling. Subjects were identified and consented for testing of their tumor samples under a protocol with Partners Healthcare Institutional Review Board approval. The initial screening cohort consisted of 180 cases of colorectal adenocarcinoma, 43 cases of pancreatic adenocarcinoma, 32 cases of gastroesophageal adenocarcinoma, 16 cases of hepatocellular carcinoma, 12 cases of biliary tract carcinoma, and four cases of small intestine adenocarcinoma. Mutational profiling of biliary tract carcinomas was further investigated retrospectively using an expanded cohort consisting of pathological specimens from 52 additional cases of cholangiocarcinoma and 23 additional cases of gallbladder carcinoma. Cholangiocarcinoma cases were designated as intrahepatic, perihilar, common bile duct, or distal bile duct based on independent review of the clinical records by two clinicians (A.X.Z., K.K.T.). Frozen cholangiocarcinoma tissue specimens used for analysis of the IDH1 and IDH2 enzymatic product were obtained from the MGH Gastrointestinal Tumor Bank by crossmatching against the list of patients studied in the expanded cohort.

Genotyping Analysis

Cancer gene mutational analysis was performed in a Clinical Laboratory Improvement Amendments–certified laboratory using formalin-fixed, paraffin-embedded tissue. Nucleic acids were extracted and common cancer gene mutations were evaluated using a custom Applied Biosystems (ABI, Carlsbad, CA) Prism SNaPshot Multiplex system, as previously reported [1]. The addition of *AKT1* p.E17K and *IDH1* p.R132C=G=H=L=S mutational assays provided screening for 130 somatic mutations across 15 cancer genes (online supplemental Table 1). Briefly, genetic regions of interest were polymerase chain reaction coamplified. Mutational hotspots were then evaluated through a single-base extension step in

which pools of allele-specific primers annealed adjacent to the target nucleotide. Extension was performed in the presence of dideoxynucleotide bases, each labeled with a distinct reporter fluorophore to identify the extended base (96°C for 30 seconds; 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 30 seconds). Labeled products were separated using an ABI Prism 3730 DNA Analyzer and the data were interpreted with GeneMapper Analysis Software (Life Technologies/Applied Biosystems, Carlsbad, CA). For the *IDH1* R132X, *IDH2* R140X, and *IDH2* R172X mutations, the primers used are listed in online supplemental Table 2.

2-Hydroxyglutarate Metabolite Detection

Frozen tumor tissue from five patients with cholangiocarcinoma was weighed and homogenized in ice cold 80% (v/v) methanol using a 1:10 (w/v) dilution of 100 mg tissue per 1 mL buffer. Covaris® Adaptive Focused Acoustics™ (Covaris, Inc., Woburn, MA) was used to generate tissue homogenates that were subsequently centrifuged at 4°C for 15 minutes. Supernatants were diluted 1:1 with 100 μL deionized water containing internal standard. Samples were then analyzed using liquid chromatography mass spectrometry as previously described [22].

Statistical Analysis

Gene mutation frequencies among different cancer types were compared using the Fisher's exact test. *p*-values $\leq$.05 were considered statistically significant. In the analysis of the prospective cohort, we adjusted *p*-values for multiple statistical tests to control the false-discovery rate at 5% [37]. No such adjustment was performed in the analysis of the validation group.

Results

Patient Characteristics

Tumors from 287 patients diagnosed across a spectrum of different gastrointestinal malignancies underwent cancer gene mutational profiling during the course of clinical management at our institution. The cohort of tumors included colorectal, pancreatic, gastroesophageal, hepatocellular, biliary tract, and small intestine carcinomas (Table 1). Following discovery of *IDH1* mutations in the initial cohort, an additional 52 cases of cholangiocarcinoma and 23 cases of gallbladder carcinoma were genotyped. The characteristics of the patients with biliary tract carcinoma evaluated in this project are listed in Table 2.

Mutational Profiling of Gastrointestinal Cancers

A high-throughput tumor genotyping platform (SNaPshot) has been developed at our institution to simultaneously query for 130 site-specific mutations distributed across 15 established cancer genes. The frequency of mutations in profiled cancer genes in the initial screen of 287 gastrointestinal cancers is summarized in Table 3. Across the overall group, the most widely mutated oncogene was *KRAS*, identified in 35% of all cases. Although *KRAS* mutations prevailed in pancreatic and colorectal cancers, they were infrequent in gastroesophageal cancers. Mutations in the *PIK3CA* gene that encodes the p110 kDa catalytic subunit of PI3K were found in 10% of all gastrointestinal cancers, with the highest prevalence in colorectal cancers. Oncogene mutations that were identified at low frequencies across multiple gastrointestinal cancer types included *BRAF* and *NRAS*, whereas *AKT1* and catenin β-1 (*CTNNB1*) mutations were exceedingly rare. The SNaPshot assay provided coverage of only the most common mutation sites within each of the tumor suppressor genes tested [1]. Within this limited scope of coverage, *TP53* was the most prevalently and widely mutated tumor suppressor gene whereas mutations in *APC* were specific to colorectal cancers (Table 3). Finally, oncogenic mutations in the tested mutational hotspot regions of *EGFR*, *FLT3*, *JAK2*, *KIT*, and *NOTCH1* were not observed.

Consistent with reports that *IDH1* and *IDH2* gene mutations are rare in tumor types other than gliomas, AML, and cartilaginous tumors [20, 36, 38–40], we identified *IDH1* mutations in only ~2% of the total gastrointestinal cohort (Table 3). However, although the relative frequency of *IDH1* mutations was extremely low in colorectal cancer (1%), it was unexpectedly found in three of 12 biliary tract carcinomas.

Table 1. Distribution of the 287 gastrointestinal cancer samples by pathologic subtype

Cancer subtype	*n*
Colorectal	
Colon adenocarcinoma	129
Rectal adenocarcinoma	51
Pancreatic	
Pancreatic adenocarcinoma	43
Gastroesophageal	
Gastric adenocarcinoma	18
Esophageal adenocarcinoma	9
Gastroesophageal junction cancer	5
Hepatic	
Hepatocellular carcinoma	16
Biliary tract	
Intrahepatic cholangiocarcinoma	10
Gallbladder carcinoma	2
Small intestine	
Small intestine adenocarcinoma	4

Detailed Analysis of *IDH1* and *IDH2* Mutations in Biliary Tract Carcinoma

To confirm this observation, a larger cohort of biliary tract carcinomas was assembled. In total, we analyzed tumors from 25 patients with gallbladder carcinoma and 62 tumors from patients with cholangiocarcinoma, including 40 intrahepatic cholangiocarcinoma patient samples and 22 extrahepatic cholangiocarcinoma patient samples (perihilar, common bile duct, or distal bile duct origin). There were no significant differences in the age, gender, baseline cancer antigen 19–9 level, or stage

Table 2. Characteristics of patients with biliary tract carcinoma ($n = 87$)

Characteristic	*n*	Percentage
Age at diagnosis, yrs		
Median	65	NA
Range	11–86	NA
Sex		
Male	40	46
Female	47	54
Site of primary disease		
Gallbladder	25	29
Intrahepatic bile duct	40	46
Extrahepatic bile duct	22	25
Baseline cancer antigen 19–9 level, U/mL		
Median	42	NA
Range	3–364,190	NA
Unknown	21	NA
Stage of disease at diagnosis		
0	1	1
I	13	15
II	14	16
III	14	16
IV	37	43
Unknown	8	9

Abbreviation: NA, not applicable.

of disease among patients with intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder carcinoma (data not shown).

A more extensive mutational evaluation was performed on these specimens, including evaluations of mutations in *IDH2* at codons R172 and R140, which were not assayed in our original panel. Diverse cancer gene mutations were identified in gallbladder carcinomas and cholangiocarcinomas in the total group of tumor samples, as shown in Figure 1 and online supplemental Table 3. The overall mutation frequency was similar in the two kinds of biliary tract tumors. The presence of *PIK3CA* mutations in gallbladder carcinomas was statistically significant when compared with the mutational profile of cholangiocarcinomas ($p = .022$), as previously reported [41].

The presence of *IDH1* mutations in biliary tract carcinomas initially identified within the clinical screening cohort of 12 patients was subsequently confirmed in the expanded cohort totaling 87 patient samples. Most notably, *IDH1* was the most frequently mutated gene in cholangiocarcinomas (eight of 62 samples, 13%) (Fig. 1), and mutations occurred exclusively in intrahepatic tumors. No such mutations were identified in 25 specimens of gallbladder carcinoma, although this difference did not reach statistical significance ($p = .099$). The primary *IDH1* mutation in cholangiocarcinomas resulted in the codon change p.R132C ($n = 5$), followed by p.R132L ($n = 2$) and p.R132G ($n = 1$) (online supplemental Table 3). Additionally, an *IDH2* p.R172W mutation was found in a single cholangiocarcinoma sample. Interestingly, neither the common glioma mutation p.R132H of *IDH1* nor the common AML mutation at codon R140 of *IDH2* was found in cholangiocarcinomas.

IDH1 and *IDH2* Mutations Predominate in Intrahepatic Cholangiocarcinomas

Cholangiocarcinoma is a heterogeneous disease that is classified by the discrete anatomical section of the bile duct in which the tumor arises. Intrahepatic tumors occur in ducts within the liver whereas extrahepatic tumors include those that arise in the perihilar ducts, the common bile duct, and the distal bile duct. There was a statistical trend ($p = .086$) for *KRAS* mutations predominating in extrahepatic versus intrahepatic cholangiocarcinoma samples (Fig. 2 and online supplemental Table 4). Most strikingly, mutations in *IDH1* and *IDH2* combined were both exclusive and predominant molecular features of intrahepatic cholangiocarcinoma samples, found in 23% (nine of 40) of cases ($p = .042$ for *IDH1*).

The clinicopathological features of the nine patients whose tumors harbored mutations in *IDH1* or *IDH2* were reviewed (online supplemental Table 5). Of these, five patients were male and four were female. Six patients had stage IV disease, two patients had stage I disease, and the disease stage of one patient was unknown. None of the patients had any known underlying risk factor associated with mutational status, including parasitic infections, primary sclerosing cholangitis, biliary duct cysts, hepatolithiasis, or thorotrast exposure. In addition, there was no association with inflammatory bowel disease, hepatitis C virus, hepatitis B virus, cirrhosis, or alcohol or tobacco use. Pathological review performed on stained tissue sections revealed that three samples were well differentiated and six were moderately differentiated. All tumors showed well-formed glands embedded within moderate to abundant stromal tissue. One tumor focally showed clear cytoplasm, and all tumors lacked both intra- and extracellular mucin. The nonneoplastic liver showed no evidence of cirrhosis in any case. Therefore, within this small group, no unique histologic or clinical features were associated with cholangiocarcinomas harboring *IDH1* or *IDH2* mutations.

2-Hydroxyglutarate Accumulation Is Associated with *IDH1* and *IDH2* Mutations

A known gain-of-function activity of mutant IDH1 and IDH2 protein is the production and subsequent tissue accumulation of the metabolite 2-hydroxyglutarate [22–24]. This biological effect of *IDH1* as well as *IDH2* mutations in cholangiocarcinoma patients was evaluated using frozen tissue obtained through surgical resection for five of the 62 cohort samples (Fig. 3). In three wild-type *IDH1* and *IDH2* samples, analysis found an average of 3.41 μg of 2-hydroxyglutarate per gram of tumor tissue (1.199 μg/g, 2.497 μg/g, and 6.534 μg/g). In a cholangiocarcinoma sample with an *IDH1* p.R132C mutation, 2-hydroxyglutarate levels were elevated 248-fold above normal (845.9 μg/g). High

Table 3. Frequency of somatic cancer gene mutations identified through routine clinical genotyping across gastrointestinal malignancies.

Gene mutation	Total cohort $n = 287$	Biliary tract $n = 12$	Colorectal $n = 180$	Gastroesophageal $n = 32$	Hepatic $n = 16$	Pancreatic $n = 43$
None identified	109[a] (38%)	8 (67%)	56 (31%)	21 (66%)	14 (88%)	9 (21%)
AKT1	2 (1%)	0	2 (1%)	0	0	0
APC	16 (6%)	0	16 (9%)	0	0	0
BRAF	20 (7%)	1 (8%)	18 (10%)	0	0	1 (2%)
CTNNB1	2[a] (1%)	0	0	0	1 (6%)	0
EGFR	0	0	0	0	0	0
FLT3	0	0	0	0	0	0
IDH1	5 (2%)	3 (25%)	2 (1%)	0	0	0
JAK2	0	0	0	0	0	0
KIT	0	0	0	0	0	0
KRAS	101[a] (35%)	0	65 (36%)	2 (6%)	0	31 (72%)
NOTCH1	0	0	0	0	0	0
NRAS	9 (3%)	0	7 (4%)	1 (3%)	0	1 (2%)
PIK3CA	29[a] (10%)	0	27 (15%)	1 (3%)	0	0
PTEN	3 (1%)	0	3 (2%)	0	0	0
TP53	63 (22%)	0	46 (26%)	8 (25%)	2 (13%)	7 (16%)

More than one mutation was identified in some samples.
[a]Small intestine cancers were included in the total cohort numbers but not listed as a subgroup because of the small number of samples.
Abbreviations: *APC*, adenomatous polyposis coli; *CTNNB1*, catenin β-1; *EGFR*, epidermal growth factor receptor; *IDH1*, isocitrate dehydrogenase 1; *JAK2*, Janus kinase 2; *KRAS*, Kirsten-ras; *PIK3CA*, phosphatidylinositol 3-kinase, catalytic, alpha; *PTEN*, phosphatase and tensin homologue deleted on chromosome ten; *TP53*, p53 tumor suppressor.

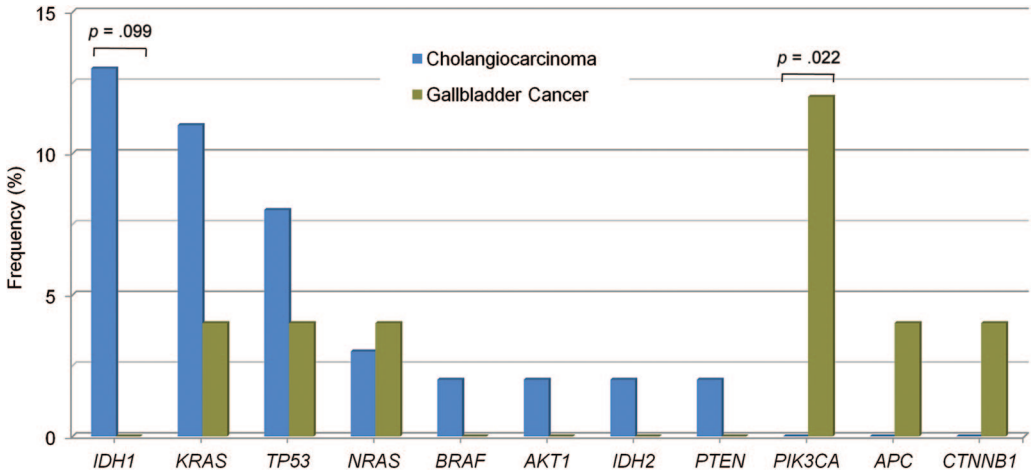

Figure 1. Mutational profile in biliary tract carcinomas. The frequency of cancer genes mutated in cholangiocarcinoma (blue bars; $n = 62$) versus gallbladder carcinoma (green bars; $n = 25$) is shown. Nucleic acids were extracted from formalin-fixed, paraffin-embedded tumor tissue and were tested for mutations using a single-base extension approach.

accumulation of 2-hydroxyglutarate (588.5 μg/g) was similarly found in a cholangiocarcinoma sample harboring the *IDH2* p.R172W mutation. Therefore, gain-of-function activity of 2-hydroxyglutarate production and accumulation is a likely biological consequence of mutant *IDH1* as well as *IDH2* in cholangiocarcinoma patients.

Discussion

This study provides the results of a broad-based clinical tumor genotyping approach that was applied across patients diagnosed with a gastrointestinal malignancy. While interrogating 130 mutations within 15 cancer driver genes using our SNaPshot platform, at least a single gene mutation was identi-

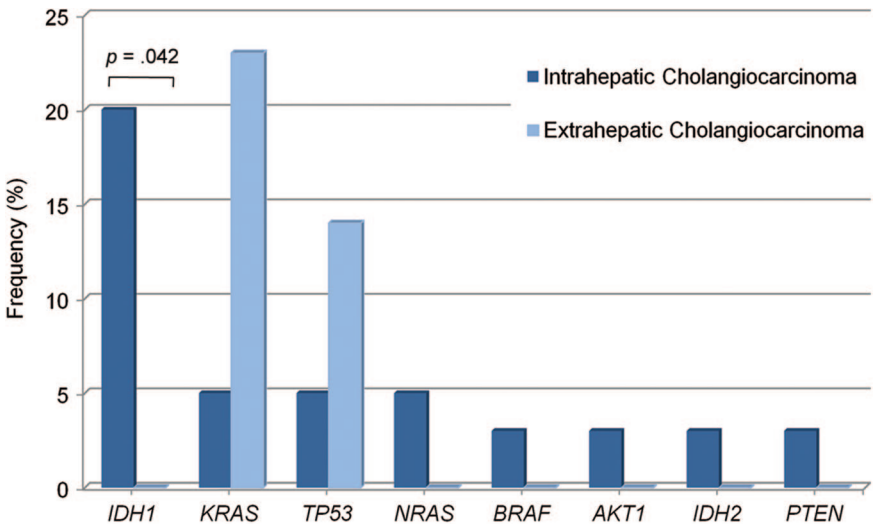

Figure 2. The distribution and frequency of profiled mutations across cholangiocarcinoma subtypes. Patient cholangiocarcinoma samples were designated as either intrahepatic (dark-blue bars; $n = 40$) or extrahepatic (light-blue bars; $n = 22$) based on surgical record and/or pathological review. Extrahepatic cholangiocarcinomas included tumors arising in the perihilar duct ($n = 11$), common bile duct ($n = 4$), or distal bile duct ($n = 7$) region.

Abbreviations: IDH, isocitrate dehydrogenase; *KRAS*, Kirsten-ras; *PTEN*, phosphatase and tensin homologue deleted on chromosome ten; *TP53*, p53 tumor suppressor.

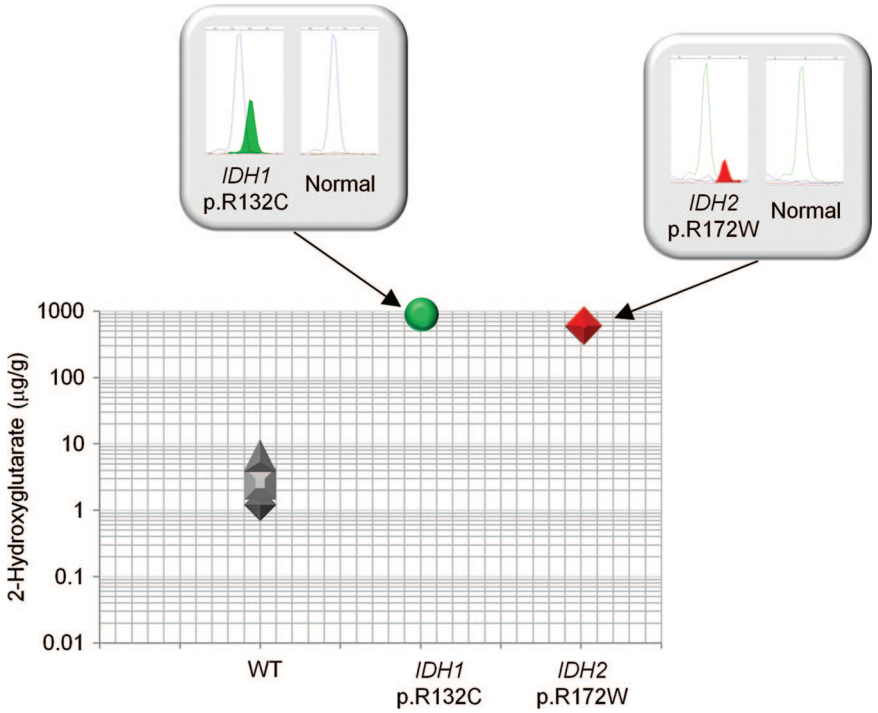

Figure 3. Mutations in the genes encoding isocitrate dehydrogenase (*IDH1* and *IDH2*) in cholangiocarcinoma are associated with 2-hydroxyglutarate metabolite accumulation. 2-Hydroxyglutarate levels were measured in frozen cholangiocarcinoma specimens using liquid chromatography mass spectrometry analysis. Three tumor samples were wild-type (WT) *IDH1* and *IDH2*, one was *IDH1* p.R132C mutation positive, and one was *IDH2* p.R172W mutation positive. SNaPshot genotyping electropherograms are shown in the boxes with the mutation peak filled.

fied in 178 of 287 tumor samples (62%). We observed that the prevalence of the tested mutations varied significantly across disease subtypes. A primary advantage of applying broad-spectrum genotyping equally across cancer groups is the potential for identifying therapeutically relevant mutations not previously known in a particular cancer type. In our study, novel *IDH1* and *IDH2* mutations in intrahepatic cholangiocarcinomas were found at high frequency (nine of 40 tumors, 23%), but they were not found in extrahepatic cholangiocarcinomas (zero of 22 tumors). The vast majority of patients with

cholangiocarcinoma present with advanced stage disease, associated with a poor prognosis. Therefore, these observations provide an important first step in assessing whether IDH1 and IDH2 abnormalities can provide a new therapeutic target that can improve clinical management of these patients.

Despite the best current therapy for patients with unresectable or metastatic cholangiocarcinoma, the median survival time remains <1 year [11, 12]. Recent phase II studies evaluating targeted agents that inhibit EGFR or mitogen-activated protein kinase/extracellular signal–related kinase kinase have demonstrated some evidence of antitumor activity in unselected biliary tract carcinoma patients [13]. Our data suggest that cholangiocarcinomas are also molecularly diverse according to sites within the biliary tract. Across the spectrum of cancer genes represented on our genotyping platform, *IDH1* and *IDH2* were the most frequently mutated genes in tumors arising in the biliary tract within the liver. Our study therefore extends the list of tumors in which mutations in *IDH1* and *IDH2* may be the driving event in tumorigenesis, including AML, central and periosteal cartilaginous tumors, and a subset of gliomas, and this may lead to new targeted therapies. Furthermore, the presence of an *IDH1* or *IDH2* mutation may assist in distinguishing intrahepatic from extrahepatic cholangiocarcinoma at the molecular level.

The mechanistic role of mutant *IDH1* and *IDH2* in tumorigenesis is unclear, but it has most recently been attributed to the gain-of-function activity of *R*(–)-2-hydroxyglutarate production through the NADPH-dependent reduction of α-ketoglutarate [22–24, 42]. The excessive accumulation of 2-hydroxyglutarate associated with *IDH1* and *IDH2* mutations inhibits the binding of α-ketoglutarate to a number of dioxygenases that require this substrate for physiological activity [43]. Prolyl-hydroxylases that function to hydroxylate and promote the degradation of the hypoxia inducible factor (HIF)-1α subunit of the HIF-1 transcription factor are α-ketoglutarate dependent, and *IDH1*-mutant gliomas have been closely associated with induced HIF-1α protein levels and target gene expression [21]. Aberrantly hypermethylated CpG islands in gene promoter regions have also been tightly associated with *IDH1* and *IDH*2 mutations in a subset of AML and glioma cases, leading to transcriptional silencing across many genes and impaired differentiation [32, 44]. These effects presumably occur through inhibition of α-ketoglutarate–dependent enzymes that control DNA and histone methylation, and may therefore promote cancer through epigenetic changes [32, 43]. Inhibition of *IDH1* and *IDH2* may become an effective strategy for cancer treatment in patients selected through tumor mutational profiling.

Conclusion

This study identified for the first time a high frequency of mutations in the isocitrate dehydrogenase genes *IDH1* and *IDH2* in cholangiocarcinomas specifically of intrahepatic origin. In two cholangiocarcinoma samples tested, these mutations were associated with accumulation of the novel 2-hydroxyglutarate metabolite. The frequency of *IDH1* and *IDH2* gene mutations was greater than the combined frequency of activating mutations in *AKT1*, *KRAS*, *NRAS*, and *BRAF* in this cancer subtype, making it the most prevalent drug targetable gene alteration in intrahepatic cholangiocarcinoma.

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